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Developmental Trajectories of Infants with Multiplex and Single-incidence Familial Risk for Autism: A Baby Siblings Research Consortium Study (in press, *JAMA Neurology*, 18-08-2019)

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Key Points

Question: How does the development of infants with multiplex and single-incidence family risk for autism spectrum disorder (ASD) differ?

Findings: In this prospective, longitudinal study that included 445 children with multiplex (*n*=80) or single-incidence (*n*=355) family risk, 68% of children from multiplex families vs. 43% of those from single-incidence families had ASD or atypical development at outcome. Non-ASD children did not differ in ASD symptoms based on family risk status, but multiplex status was associated with lower cognitive abilities by age 3.

Meaning: Infants with a multiplex family history of ASD should be monitored early and often and referred for early intervention services at the first sign of concern.

Abstract

Importance: Autism spectrum disorder (ASD) is a neurodevelopmental disorder associated with different genetic etiologies. Prospective examination of familial-risk infants informs understanding of developmental trajectories preceding ASD diagnosis, potentially improving early detection.

Objective: Compare outcomes and trajectories associated with varying familial risk for ASD across first 3 years of life.

Design and Setting: This longitudinal, prospective observational study included data from 11 sites in the Baby Siblings Research Consortium (BSRC) database. Data collected between 20XX-20XX. Analyses conducted in 2018.

Participants: From initial sample of 1,008 infants from BSRC database, 573 removed due to missing necessary data, diagnostic discrepancies, or having only one older sibling. 435 younger siblings of children with ASD were included; 355 came from *single-incidence* families (1 sibling with ASD and 1+ sibling without ASD) and 80 from *multiplex* families (2+ siblings with ASD). Participants contributed data at multiple time points from 6-36 months old. Multiplex and single-incidence groups did not differ on major demographics.

Exposure: Number of ASD-siblings.

Main Outcomes and Measures: Outcomes included ASD symptoms, cognitive abilities, and adaptive skills. Diagnosis (*ASD/no-ASD*) was given at 36-month outcome. No-ASD group further classified as *atypical* (developmental delays and/or social-communication concerns) or *typical* for some analyses. Generalized linear mixed models examined developmental trajectories by ASD outcome and familial-risk group.

Results: In the 435 analyzed participants (age range at outcome: 32-43 months; 57% male),

children from multiplex families were more likely than those from single-incidence families to be classified as ASD (36% vs. 16%) and less likely as typical (33% vs. 57%), with similar rates of atypical classifications (31% vs. 27%). No differences in ASD symptoms between multiplex and single-incidence groups over time, after controlling for ASD outcome. During infancy, differences in cognitive and adaptive abilities observed based upon ASD outcome, but not familial-risk. At 36 months, multiplex/no-ASD group had lower cognitive abilities than single-incidence/no-ASD group, and multiplex had lower adaptive abilities than single-incidence, after controlling for ASD outcome.

Conclusions and Relevance: Infants with a multiplex family history of ASD should be monitored early and often and referred for early intervention at first sign of concern. Direct examination of genetic contributions to neurodevelopmental phenotypes in infants with familial risk for ASD is needed.

Keywords: Multiplex; familial risk; autism

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and the presence of restricted and repetitive patterns of behavior[1]. Recent estimates indicate an ASD prevalence of 1 in 59 children and a typical age of diagnosis of 4 years old[2]. Converging evidence suggests that there are multiple genetic pathways to ASD[3,4]. One genetic risk group that has been studied widely includes infants with at least one older sibling with ASD (*familial-risk*). Prospective studies of these infants have helped to characterize the early emergence of developmental differences associated with later ASD diagnosis, with the identification of these early signs ultimately improving early screening and intervention efforts[5].

To overcome the challenge of small sample sizes and to facilitate scientific collaboration in prospective studies of familial-risk infants, the Baby Siblings Research Consortium (BSRC) was formed. BSRC researchers have combined data from common measures across many sites to answer clinically-relevant questions about early manifestations of ASD. BSRC research indicates that nearly 20% of familial-risk infants will meet criteria for ASD at age 3[6,7] and another approximately 20% will show other developmental atypicalities (e.g., developmental delays, subclinical ASD symptoms)[8,9]. One key question arising from these prospective studies is whether neurodevelopmental outcomes vary based on genetic risk, with variability in risk defined by the number of siblings with ASD. *Multiplex* ASD (2+ older ASD-siblings) is more commonly associated with the additive risk of common genetic variants and inherited copy number variants (CNVs)[10.11], while *single-incidence* ASD (one older ASD-sibling) is more often caused by rare de novo CNVs and mutations[11].

Prior BSRC studies have shown that 60% of male and 30% of female children with

multiplex family risk have ASD compared to nearly 30% of male and 10% of female children with single-incidence family risk[6]. Profile analyses of these infants has indicated that multiplex status is associated with decreased cognitive scores, but no difference in ASD symptoms[6]. Family-based studies have demonstrated that the non-ASD siblings of children with ASD from multiplex families have a higher level of subclinical ASD symptoms[12,13], while the degree to which cognitive abilities differ among non-ASD siblings based on familial-risk status is less clear[14]. No studies have analyzed differences in developmental trajectories in infancy based on multiplex versus single-incidence status.

Using the BSRC database, we comprehensively examined categorical distinctions and developmental trajectories in social-communication, cognitive, and adaptive skills associated with different levels of familial risk across the first 3 years of life. We sought to answer three primary questions: (1) How do rates of typical, atypical (non-ASD), and ASD outcomes differ between infants from multiplex and single-incidence families? (2) When and how do developmental trajectories of ASD symptoms, cognitive ability, and adaptive skills across the first 3 years of life diverge based on familial-risk status and ASD diagnostic outcome? (3) For children without ASD, how do the phenotypic profiles differ at 3-year outcome based on familial-risk status? We expected greater impairment in infants from multiplex families versus single-incidence families, with higher rates of ASD overall, as well as lower developmental and adaptive abilities, and higher subclinical ASD symptoms in non-ASD children. Results of these analyses can help guide clinicians in earlier and more informed developmental screening and monitoring of infants from multiplex families.

Method

Participants

Out of an initial sample of 1,008 potential participants from the BSRC database, 435 younger siblings of children with ASD who were enrolled in longitudinal studies across 10 BSRC sites met inclusion criteria (partially overlapping with previous BSRC samples[6,7]). Children in the *multiplex* group had 2+ older siblings with ASD. Unlike previous BSRC studies[6,7], children in the *single-incidence* group had a single older sibling with ASD and 1+ older sibling without ASD. Confirmation of older sibling diagnoses varied by study site.

Participants were removed due to missing required outcome data (*n*=110), discrepancies between ADOS score and diagnosis (*n*=15), missing information on older siblings (*n*=8), having only one older sibling (*n*=404), or multiple siblings from the same family (*n*=36). When more than one child from a family participated in the study, only the youngest child was included to maximize information on older siblings.

Groups were comparable with regard to demographic characteristics (Table 1). The multiplex group had larger families than the single-incidence group. IRB approval and written informed consent for all participants was obtained within each study site.

Measures

ASD symptoms were measured at 18, 24, and 36 months of age (time points varied by study site) using the Autism Diagnostic Observation Schedule (ADOS)[15], an observational measure of social-communication and repetitive behaviors. The ADOS yields a Calibrated Severity Score (CSS) ranging from 1-10[16,17]. The CSS-Overall score was used in longitudinal analyses. The Social Affect (SA) and Restricted, Repetitive Behavior (RRB) subscale scores were examined in outcome analyses. The Autism Diagnostic Interview-Revised (ADI-R)[18], a parent interview, was collected at 36 months in a subset of infants and used as a secondary indicator of ASD symptoms in outcome analyses.

Cognitive abilities were measured at 6, 9, 12, 15, 18, 24, and 36 months (varied by study site), using the Mullen Scales of Early Learning (MSEL)[19]. The MSEL examines Visual Reception, Fine Motor, Receptive Language, and Expressive Language, which yield *t*-scores (*M*=50, *SD*=10). An Early Learning Composite (ELC) is also calculated, yielding a standard score (*M*=100, *SD*=15) representing a child's overall cognitive ability relative to peers. The ELC was used in longitudinal analyses. Subscale scores were analyzed in outcome analyses.

Adaptive skills were assessed at 6, 9, 12, 15, 18, 24, and 36 months (varied by study site) in a subset of infants using the Vineland Adaptive Behavior Scales-Second Edition (Vineland-II)[20], a parent-report measure. The Vineland-II assesses Communication, Daily Living Skills, Socialization, and Motor Skills, which produce standard scores. The Adaptive Behavior Composite (ABC) is computed from the first three domains, yielding a standard score representing an individual's overall adaptive ability relative to peers. The ABC was utilized in longitudinal analyses. Subscale scores were examined in outcome analyses.

Clinical outcomes were determined following the 36-month assessment. Children were classified as *ASD* (*n*=86; vs. *no-ASD*, *n*=349) if they had a clinical best estimate diagnosis of ASD by expert clinicians *and* an ADOS score at or above the clinical threshold (CSS ≥ 4)[7]. For categorical analyses only, the no-ASD group was split into a *typical* (*n*=227; MSEL ELC ≥ 85 and ADOS CSS < 3) and *atypical* group (*n*=122; MSEL ELC < 85 and/or ADOS CSS ≥ 3)[8,21]. Within the atypical group, 25.9% fell into this group due to lower cognitive scores, 64.7% due to elevated ADOS scores, and 9.5% due to both factors (Table 1).

Statistical Analyses

Longitudinal trajectories of primary outcome variables (ADOS CSS-Overall, MSEL ELC, Vineland-II ABC) were modeled using generalized linear mixed models (GLMM) with

main effects of ASD outcome (ASD vs. no-ASD), familial-risk status (multiplex vs. single-incidence), and time, along with their two-way and three-way interactions. Subject-specific and site-specific random intercepts were included to model dependency due to repeated measures within subjects and sites. MSEL and Vineland-II scores were modeled employing an identity link, while ADOS scores were modeled using a negative binomial GLMM with a log-link. Time was modeled as a class variable for ADOS (at 18, 24, and 36 months), with a broken line model allowing for a slope change at 18 months for MSEL and linearly for Vineland-II (where a slope change at 18 months was non-significant). Two-way and three-way interactions between ASD outcome, familial-risk status, and time were found significant in models for Vineland-II and MSEL; however, the final GLMM for ADOS only contained the significant two-way interaction between ASD outcome and time.

According to the interactions found significant and our hypotheses, we conducted 6 contrasts for MSEL and Vineland-II data at pre-selected time points to evaluate group mean differences between: (1) ASD and no-ASD single-incidence, (2) ASD and no-ASD multiplex, (3) no-ASD multiplex and single-incidence, (4) ASD multiplex and single-incidence, (5) (ASD multiplex – no-ASD multiplex) and (ASD single-incidence – no-ASD single-incidence), (6) multiplex and single-incidence. Contrasts were conducted at 6, 12, 24, and 36 months for MSEL and 12, 24 and 36 months for Vineland-II (time points with most observations). For the final ADOS model, we conducted contrasts between: 1) ASD and no-ASD groups at 18, 24, and 36 months, and 2) multiplex and single-incidence groups (ages collapsed). We used false discovery rate (FDR)[22] at .05 to adjust for multiple comparisons (46 contrasts).

GLMMs account for correlations between repeated measures within subjects, allowing for fixed and time-varying covariates and automatically handling missing data, thereby

producing unbiased estimates as long as observations are missing at random. Accordingly, all available observations from each subject were utilized in modeling via GLMM.

Results

36-month outcome classifications based on familial-risk status

Outcome classifications significantly differed based on familial-risk status, $\chi^2(2, N=435)=21.10$, $p<.001$. The multiplex group was more likely than the single-incidence group to be classified as ASD (36.3% vs. 16.1%), $p<.001$, less likely to be classified as typical (32.5% vs. 56.6%), $p<.001$, and had similar levels of atypical classifications (31.3% vs. 27.3%), $p=.493$.

Developmental trajectories based on familial-risk status and ASD outcome

Results from the final GLMMs are summarized below. See Figure 1 for depictions of modeled developmental trajectories, Table 2 for detailed sample size information, and Table 3 for contrast results (eFigure2 presents raw trajectories).

***ASD symptoms*.** ASD symptoms differed between the ASD and no-ASD groups, regardless of familial-risk status, at 18, 24, and 36 months. As expected, children with ASD outcomes showed higher levels of ASD symptoms than children without ASD beginning at 18 months. There were no differences in ASD symptoms between the multiplex and single-incidence groups, after controlling for ASD outcome.

***Cognitive abilities*.** Within the single-incidence group, children with ASD outcomes had lower cognitive abilities than no-ASD children at 6, 12, 24, and 36 months. In the multiplex group, the ASD and no-ASD groups did not differ at 6 or 12 months; instead, differences emerged at 24 months, with the ASD group demonstrating lower cognitive abilities than the no-ASD group at 24 and 36 months. Within the no-ASD group, the multiplex group had lower cognitive abilities than the single-incidence group at 36 months; cognitive abilities did not differ

based on familial-risk status among no-ASD children at earlier ages. In the ASD group, cognitive abilities did not differ between multiplex and single-incidence groups. There was, however, an overall difference in cognitive abilities between multiplex and single-incidence groups (ASD + no-ASD contrast) at 36 months. Finally, the difference in cognitive abilities among ASD and no-ASD children differed between the multiplex and single-incidence groups (ASD – no-ASD contrast) at 6 months. As depicted in Figure 1b, children with ASD outcomes had lower cognitive abilities than those without ASD within the single-incidence group at 6 months, while multiplex children had similar abilities at this age regardless of ASD outcome.

***Adaptive skills*.** Within the single-incidence group, children with ASD outcomes had lower adaptive abilities than no-ASD children at 12, 24, and 36 months. Within the multiplex group, children with and without ASD outcomes showed similar levels of adaptive abilities at 12 months, which then diverged at 24 and 36 months. The multiplex and single-incidence groups did not, however, differ significantly within the ASD and no-ASD groups. Likewise, overall familial-risk group differences were mostly non-significant. At 36 months, there were overall differences based on familial-risk status; the multiplex group had lower adaptive abilities than the single-incidence group.

36-month developmental profiles based on familial-risk status in no-ASD children

See Table 4 for descriptive information and statistical results (presented in eFigure1). Results are reported with and without correction for multiple comparisons (13 contrasts). No-ASD children from multiplex and single-incidence groups showed similar levels of social-communication skills and RRBs on the ADOS and ADI-R, and communication, socialization, daily living, and motor skills on the Vineland-II. On the MSEL, however, the multiplex group had lower visual reception and receptive language scores than the single-incidence group; the

difference in receptive language survived FDR correction.

Discussion

This longitudinal investigation indicated areas of similarity and difference associated with varying levels of familial risk for ASD and developmental delay.

Rates of ASD

Children from multiplex families were more than twice as likely to have ASD outcomes as those from single-incidence families. While 57% of the children with only one older sibling with ASD were typically developing at age 3, only 33% of the children with multiple older siblings with ASD were typically developing at outcome. This finding highlights the first and most important clinical finding of this study: infants with a strong family history of ASD need to be monitored early and often, and they should be referred for early intervention services at the first sign of concern.

Developmental trajectories

Longitudinal analyses suggest that group differences over time in ASD symptoms, cognitive abilities, and adaptive skills were mainly attributable to ASD outcome rather than familial-risk status. This was particularly true for ASD symptoms, which differed only based upon ASD outcome beginning at 18 months. Within the single-incidence group, children with ASD outcomes consistently demonstrated lower cognitive abilities than children without ASD beginning at 6 months and adaptive abilities beginning at 12 months (earliest ages contrasted). Conversely, multiplex infants showed similar levels of cognitive and adaptive abilities at earlier ages, regardless of ASD outcome, and did not diverge until the second year of life. Multiplex children with ASD outcomes demonstrated a sharp decline in standard scores on measures of early cognitive and adaptive skills in the second and third years of life, reflecting slower growth

in these developmental abilities. Interestingly, neuroimaging studies of familial-risk infants have identified altered trajectories of brain development in the first year, particularly in cortical surface area and neural connectivity[23,24]. These studies have not distinguished infants based on multiplex vs. single-incidence status, but they support the hypothesis that genetic risk factors lay a foundation for early changes in brain structure and function, which may then cumulatively disturb learning and adaptive behaviors leading to difficulties making expected developmental gains. These neurobiological changes may truly precede behavior; alternatively, our standardized behavioral measures may lack sensitivity to discern subtle changes in development in the first year of life. Clinically, these results suggest that it may be more challenging to distinguish infants with ASD vs. no-ASD outcomes behaviorally in the context of multiplex status during infancy and early toddlerhood. Further research longitudinally examining biomarkers of risk early in life is critically needed to determine which infants are most likely to require pre-emptive intervention in this population[25,26].

Profile analyses

We also detected subtle differences and remarkable similarities between multiplex and single-incidence children without ASD at outcome. Non-ASD children from multiplex and single-incidence families did not differ in their observed or parent-reported levels of ASD symptoms at age 3. This was somewhat surprising given previous research suggesting subclinical ASD symptoms in family members of individuals with ASD (i.e., broader autism phenotype), particularly families with multiple affected individuals[13,27]. It is possible that our ASD symptom measures, which were designed as clinical diagnostic tools, were not sensitive enough to detect subtle differences in social-communication and repetitive behavior.

We did, however, detect differences in cognitive abilities at age 3. This finding was primarily explained by differences in receptive language, and, to a lesser degree, nonverbal cognitive skills, with no differences in broadly-measured expressive language abilities found between the non-ASD multiplex and single-incidence groups. These results are largely consistent with previous research finding deficits in verbal IQ in the unaffected siblings of multiplex but not single-incidence families[14]. The likely risk factors for having multiple children with ASD, such as shared genetic variation, vulnerability to genetic mutations, or complex gene x environment interactions (e.g., in utero environment) may impact brain development in a more distributed, global way, which then impacts overall development, rather than networks that are more specific to ASD. These findings strongly speak to the need for large, collaborative efforts to examine brain development, genetics, and gene-environment interactions in at-risk infants to understand the neurobiological mechanisms underlying these differences in developmental trajectories in behavior.

Strengths and limitations

Our study uniquely leveraged a rich dataset collected from multiple expert sites to prospectively examine differences associated with multiplex familial-risk status and diagnostic outcome in a large cohort of infants with elevated genetic risk for ASD. Although the sample size was quite large for a study of this kind, the prospective nature of the study led to uneven and occasionally small groups that disallowed firm conclusions in some areas of interest. For instance, the multiplex group was smaller, so comparisons within this group (e.g., ASD vs. no-ASD) were less powered than those within the single-incidence group. Given the longitudinal, multi-site design, there was also some inconsistency among study sites in the ages at which different measures were collected, and missing data. Statistical models that account for missing

data and site differences helped to attenuate possible negative effects. The use of already collected data across multiple sites also required us to choose common broad-based measures that, while highly clinically relevant and well-validated, may not have been sensitive enough to detect more subtle differences between the non-ASD children. Additionally, as is the case across the ASD-sibling literature[28], many of the children in the sample had relatively high cognitive scores and came from predominantly Caucasian and highly-educated families, so these results may not represent the larger population of children with ASD. The most substantial limitation is the lack of genomic data in these infants, which would inform our hypotheses about genetic factors contributing to developmental differences. There are several ongoing studies that are seeking to fill this gap, and this study reinforces the value in future integration of genetics with behavioral assays to better understand the link between genetic risk and neurodevelopment in infancy.

Conclusions

Children from multiplex families are more than twice as likely to meet criteria for ASD at age 3 than children from single-incidence families. Prospectively, single-incidence infants begin to show developmental differences based on later ASD diagnosis by 6 months of age, while multiplex infants with and without ASD outcomes do not differ until the second year of life. Among unaffected children, multiplex risk is associated with lower cognitive abilities, but similar levels of ASD symptoms. Results support the need for direct examination of genetic contributions to neurodevelopmental phenotypes in infants with multiplex and single-incidence family risk for ASD. Given their very high risk for ASD and other neurodevelopmental challenges, infants with a strong family history of ASD should be monitored early and often and referred for early intervention at the first sign of developmental concern.

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Concept and design: McDonald, Jeste.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: McDonald, Jeste, Senturk.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: McDonald, Senturk, Scheffler.

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Table 1. Participant information by familial risk and outcome group

Variable	**Single-incidence (*n*=355)**	**Multiplex (*n*=80)**	***p***
Sex (male) *n* (%)	200 (56.3)	46 (57.5)	.85
Race/ethnicity (non-Caucasian) *n* (%)	86 (24.3)	14 (17.5)	.36
Maternal education (college or higher) *n* (%)	233 (65.7)	53 (66.7)	.92
Maternal age at birth (years) *M (SD)*	34.86 (4.82)	34.57 (4.87)	.69
Paternal age at birth (years) *M (SD)*	37.37 (5.81)	37.35 (6.26)	.98
Number of children in family *M (SD)*	3.46 (.79)	3.83 (1.35)	.02
Number of ASD siblings *M (SD)*	1 (0)	2.13 (.44)	<.001
Age first seen (months) *M (SD)*	6.90 (4.24)	7.23 (4.14)	.53
Age at outcome (months) *M (SD)*	37.28 (1.63)	37.48 (1.92)	.34
36-month outcome variable*	**Typical (*n*=227)**	**Atypical (*n*=122)**	**ASD (*n*=86)**
ASD symptoms (ADOS) M (SD)			
Overall	1.30 (.46)	3.52 (1.77)	6.85 (1.78)
Social Affect	1.64 (.77)	3.85 (2.08)	6.67 (1.83)
RRB	3.10 (2.37)	5.07 (2.45)	7.58 (1.91)
Cognitive abilities (MSEL) M (SD)			
Early Learning Composite	110.49 (14.37)	96.90 (19.84)	81.06 (24.42)
Visual Reception	61.25 (10.31)	53.14 (15.14)	42.54 (18.84)
Fine Motor	52.90 (12.65)	44.73 (12.81)	36.12 (12.75)
Receptive Language	52.77 (8.84)	46.61 (9.86)	38.52 (14.31)
Expressive Language	54.14 (7.82)	47.84 (10.94)	39.81 (13.56)
Adaptive skills (Vineland-II) M (SD)			
Adaptive Behavior Composite	94.95 (12.11)	90.37 (13.84)	79.14 (13.73)
Communication	100.90 (13.18)	96.12 (14.16)	85.22 (16.32)
Daily Living Skills	94.95 (11.41)	89.95 (15.90)	80.55 (14.04)
Socialization	97.66 (12.68)	92.97 (12.85)	79.59 (13.21)
Motor Skills	94.88 (12.06)	92.00 (12.71)	84.83 (12.78)

Note. Group differences between categorical variables assessed using chi-square tests, and continuous variables using t-tests. ADOS calibrated severity score (1-10), Vineland-II composite

and domain standard scores (*M*=100, *SD*=15), MSEL composite standard score (*M*=100, *SD*=15) and subscale t-scores (*M*=50, *SD*=10) presented. *Groups differed on all outcome variables.

Table 2. Number of participants with data by age, measure, and group status

Age (mos)	Single-incidence/No-ASD	Single-incidence/ASD	Multiplex/No-ASD	Multiplex/ASD
Total in sample	298	57	51	29
ADOS				
18	214	39	36	23
24	260	54	44	27
36	298	57	51	29
MSEL				
6	175	29	28	13
9	48	11	14	5
12	249	38	41	23
15	57	16	11	7
18	113	19	18	15
24	266	49	48	27
36	294	56	49	27
Vineland-II				
6	38	9	5	2
9	38	9	4	3
12	148	26	21	14
15	42	13	7	3
18	177	28	29	17
24	187	37	28	18
36	210	39	30	17
ADI-R				
36	136	37	28	15

Note. GLMM models used all available data to inform estimates.

Table 3. GLMM contrast results

Group	Contrast	Age (mos)	Estimate (SE)	*df*	*t*	*p*	*f*
Observed ASD symptoms (ADOS)							
-	ASD vs. No-ASD	18	.726 (.081)	693	8.949	<.001	.340
-	ASD vs. No-ASD	24	.776 (.071)	693	10.953	<.001	.416
-	ASD vs. No-ASD	36	1.158 (.065)	693	17.938	<.001	.681
-	Multiplex vs. Single-incidence	-	.093 (.056)	693	1.667	.177	.057
Cognitive abilities (MSEL)							
Single-incidence	ASD vs. No-ASD	6	-6.524 (2.665)	1304	-2.448	.042	.068
Single-incidence	ASD vs. No-ASD	12	-10.154 (2.015)	1304	-5.038	<.001	.140
Single-incidence	ASD vs. No-ASD	24	-17.527 (2.043)	1304	-8.578	<.001	.238
Single-incidence	ASD vs. No-ASD	36	-25.012 (2.349)	1304	-10.649	<.001	.295
Multiplex	ASD vs. No-ASD	6	5.672 (4.297)	1304	1.320	.287	.037
Multiplex	ASD vs. No-ASD	12	-5.893 (3.167)	1304	-1.861	.126	.052
Multiplex	ASD vs. No-ASD	24	-18.844 (3.182)	1304	-5.923	<.001	.164
Multiplex	ASD vs. No-ASD	36	-21.614 (3.808)	1304	-5.676	<.001	.157
No-ASD	Multiplex vs. Single-incidence	6	-2.484 (2.669)	1304	-.931	.438	.026
No-ASD	Multiplex vs. Single-incidence	12	-.649 (2.048)	1304	-.317	.785	.009
No-ASD	Multiplex vs. Single-incidence	24	-1.558 (2.111)	1304	-.738	.505	.020
No-ASD	Multiplex vs. Single-incidence	36	-7.045 (2.473)	1304	-2.848	.015	.079

ASD	Multiplex vs. Single-incidence	6	9.712 (4.307)	1304	2.255	.053	.062
ASD	Multiplex vs. Single-incidence	12	3.612 (3.162)	1304	1.142	.359	.031
ASD	Multiplex vs. Single-incidence	24	-2.875 (3.152)	1304	-.912	.438	.025
ASD	Multiplex vs. Single-incidence	36	-3.647 (3.741)	1304	-.975	.434	.027
ASD – No-ASD	Multiplex vs. Single-incidence	6	12.196 (5.061)	1304	2.410	.044	.067
ASD – No-ASD	Multiplex vs. Single-incidence	12	4.261 (3.760)	1304	1.133	.359	.031
ASD – No-ASD	Multiplex vs. Single-incidence	24	-1.317 (3.786)	1304	-.348	.779	.010
ASD – No-ASD	Multiplex vs. Single-incidence	36	3.398 (4.479)	1304	.759	.503	.021
ASD + No-ASD	Multiplex vs. Single-incidence	6	3.614 (2.536)	1304	1.425	.255	.039
ASD + No-ASD	Multiplex vs. Single-incidence	12	1.481 (1.887)	1304	.785	.498	.022
ASD + No-ASD	Multiplex vs. Single-incidence	24	-2.216 (1.901)	1304	-1.166	.359	.032
ASD + No-ASD	Multiplex vs. Single-incidence	36	-5.346 (2.246)	1304	-2.381	.045	.066
Adaptive skills (Vineland-II)							
Single-incidence	ASD vs. No-ASD	12	-7.587 (1.756)	843	-4.321	<.001	.149
Single-incidence	ASD vs. No-ASD	24	-9.816 (1.523)	843	-6.430	<.001	.221
Single-incidence	ASD vs. No-ASD	36	-12.046 (1.941)	843	-6.207	<.001	.216

Multiplex	ASD vs. No-ASD	12	-2.624 (3.012)	843	-0.871	.453	.030
Multiplex	ASD vs. No-ASD	24	-9.381 (2.502)	843	-3.750	.001	.129
Multiplex	ASD vs. No-ASD	36	-16.138 (3.316)	843	-4.867	<.001	.168
No-ASD	Multiplex vs. Single-incidence	12	-4.140 (1.932)	843	-2.143	.068	.074
No-ASD	Multiplex vs. Single-incidence	24	-3.669 (1.612)	843	-2.276	.053	.078
No-ASD	Multiplex vs. Single-incidence	36	-3.199 (2.123)	843	-1.506	.234	.052
ASD	Multiplex vs. Single-incidence	12	.823 (2.902)	843	0.284	.794	.010
ASD	Multiplex vs. Single-incidence	24	-3.234 (2.448)	843	-1.321	.287	.046
ASD	Multiplex vs. Single-incidence	36	-7.291 (3.202)	843	-2.277	.053	.078
ASD – No-ASD	Multiplex vs. Single-incidence	12	4.963 (3.487)	843	1.423	.255	.049
ASD – No-ASD	Multiplex vs. Single-incidence	24	.435 (2.931)	843	.149	.882	.005
ASD – No-ASD	Multiplex vs. Single-incidence	36	-4.092 (3.842)	843	-1.065	.388	.037
ASD + No-ASD	Multiplex vs. Single-incidence	12	-1.653 (1.743)	843	-.951	.437	.033
ASD + No-ASD	Multiplex vs. Single-incidence	24	-2.555 (1.513)	843	-1.689	.176	.058
ASD + No-ASD	Multiplex vs. Single-incidence	36	-5.245 (1.921)	843	-2.730	.020	.076

Note. Reported *p*-values are FDR corrected.

Table 4. Detailed comparison of 36-month outcome data across familial-risk groups in no-ASD children

Variable *M (SD)*	**Single-incidence**	**Multiplex**	p^{raw}	p^{FDR}	***d***
Cognitive abilities (MSEL)					
Visual Reception	59.14 (12.26)	54.70 (14.85)	.050	.224	.33
Fine Motor	50.66 (13.03)	46.96 (14.32)	.069	.224	.27
Receptive Language	51.80 (9.91)	47.61 (8.80)	.003	.039	.45
Expressive Language	52.28 (9.38)	50.39 (9.89)	.195	.293	.20
Observed ASD symptoms (ADOS)					
Social Affect	2.38 (1.74)	2.63 (1.68)	.346	.409	.15
RRB	3.68 (2.58)	4.41 (2.48)	.059	.224	.29
Reported ASD symptoms (ADI-R)					
Social Interaction	2.66 (2.30)	3.82 (3.84)	.133	.293	.37
Communication	2.39 (2.50)	3.29 (3.47)	.203	.293	.30
RRB	.73 (1.39)	1.29 (1.86)	.138	.293	.34
Adaptive skills (Vineland-II)					
Communication	99.57 (13.61)	96.03 (14.33)	.170	.293	.25
Daily Living Skills	93.47 (13.43)	90.24 (13.71)	.229	.298	.24
Socialization	96.17 (12.03)	93.85 (17.62)	.464	.503	.15
Motor Skills	93.90 (11.62)	93.07 (16.81)	.799	.799	.06

Note. Both uncorrected (p^{raw}) and FDR-corrected (p^{FDR}) *p-values reported.*

Figure 1. Developmental trajectories of (a) ASD symptoms, (b) cognitive abilities, and (c) adaptive skills based on family risk status and ASD diagnostic outcome

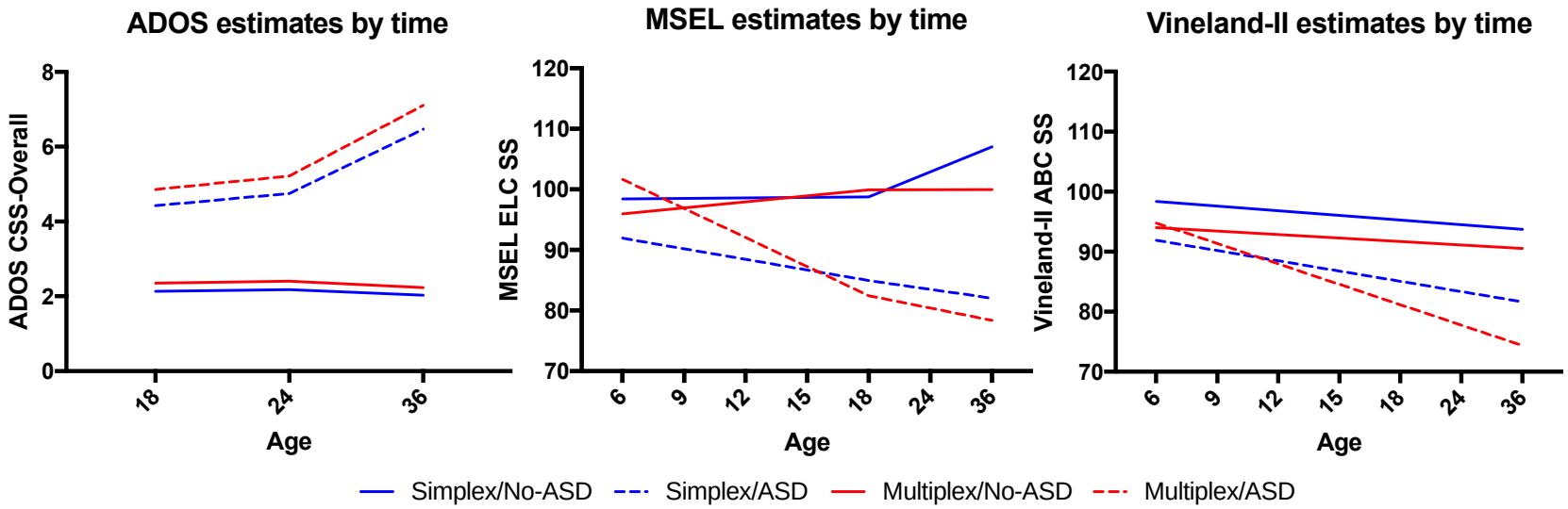

Figure 1 caption. Depiction of results from Generalized Linear Mixed Models. ADOS measured ASD symptoms, MSEL measured cognitive abilities, and Vineland-II measured adaptive skills. ADOS=Autism Diagnostic Observation Schedule. CSS=Calibrated Severity Score. MSEL=Mullen Scales of Early Learning. ELC=Early Learning Composite. SS=Standard Score. ABC=Adaptive Behavior Composite.